云 南 植 物 研 究 1994; 16 (4): 417—423
Acta Botanica Yunnanica

乌奴龙胆中五个新的环烯醚萜甙

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摘要 从藏药乌奴龙胆(*Gentiana urnula* Smith)(龙胆科)的全草中分离到 5 个新的环烯醚萜甙, 命名为乌奴龙胆甙(gentiournoside)A—E; 它们的结构主要通过光谱分析得以确定。其中, 乌奴龙胆 A—C 是二聚环烯醚萜甙, 而乌奴龙胆甙 D 和 E 为马钱素型的环烯醚萜甙, 所有这些化合物的分子中都具有一个 2, 3-二羟基苯甲酰基或其衍生物的取代基。

关键词 乌奴龙胆, 龙胆科, 环烯醚萜甙, 乌奴龙胆甙 A—E

FIVE IRIDOIDAL GLYCOSIDES FROM GENTIANA URNULA

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Abstract Five new iridoidal glycosides, gentiournosides A—E were isolated from the whole herb of *Gentiana urnula*. Their structures were elucidated mainly by spectral methods. Among which gentiournosides A—C are bis-iridoidal glycosides while gentiournosides D and E are loganin type ones. It is noted that all the glycosides possess a 2,3-dihydroxy benxoyl unit or its derivative in the molecule.

Key words *Gentiana urnula*, Gentianaceae, Iridoidal glycosides, Gentiournosides A—E

Gentiana urnula Smith is a small gentianaceous gerb distributed in the high altitude region of western China. It is used in Tibetan traditional medicine as an antipyretic and an antidote as well as for the treatment of thrombus, dysentery and sore-throat [1]. As part of our study on the chemistry of iridoidal glycosides of Gentianaceae, we examined the n-butanol soluable fraction of a methanol extract of the whole herb of *Gentiana urnula*, which led to the isolation of five new iridoidal glycosides designated gentiournosides A—E(1—5).

Gentiournosides A(1) showed UV maximum peaks at 222,238 and 322(lgε3.47, 3.35 and 2.40, respectively) and IR absorption bands at 3400(br.), 1700, 1670, 1625, 1580 and 1460, which was suggestive of the skeleton of an iridoidal glycoside [2] and a substituent of an aromatic group. Its molecular formula $C_{40}H_{52}O_{22}$ was determined by the negative FAB mass spectrum, in which a quasi-molecular ion peak exhibited at m / z 883 in combination with the ^{13}C NMR(DEPT) spectrum. Comparison of the ^{13}C and ^{1}H NMR signals of **1** with reported data(Table 1) of loganin(**7**) [3], 7-O-acylated loganin, periclymenoside [3] and 7-O-acetylsecologanol (**8**) [4] revealed that it should have partial structures of 7-O-acylated loganin

1993-12-13 收稿, 1994-02-17 修回

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(patr 'a'), 7-O-acylated secologanol (part 'b') and an aromatic substituent. This aromatic substituent was proved to be 2,3-dihydroxy benzoyl group by comparison of the ^1H and ^{13}C NMR signals with those of depressoside from *G. depressa* [5] in which such an unit is present. The linkage sequence of the three units were established as follows. On mild alkaline hydrolysis with 0.5% Et_2NH-MeOH, **1** yielded compound **6** which showed a quasi-molecular ion peak at 747[M($C_{33}H_{48}O_{19}$)-H]$^-$ in the negative FAB mass spectrum, indicating it was the de-aromatic product of **1.** Analysis of the ^{13}C and ^1H NMR signals of **6** led to a conclusion that part 'a' was attached to the hydroxy group of C-7 of part 'b'. Thus, the aromatic group should be linked to the hydroxy group of C-7 of part 'a' in **1** , which resulted in a downfield shift of α-carbon (4.96 ppm) and upfield shifts of β-carbons(-2.24 ppm and -0.92 ppm for C-6 and C-8, respectively) when compared against **6.** In addition, a ^1H-^1H COSY of **1** was preformed and most proton signals were unequivocally assigned in the light of each coupling system (Table 2). Based on the above evidence, the structure of **1** was established as shown in Figure 1.

Gentiournoside B(2) displayed a quasi-molecular ion peak at 1045[M($C_{46}H_{62}O_{27}$-H]$^-$ in the negative FAB mass spectrum. On comparison of the ^{13}C and ^1H NMR spectra with those of **1, 2** showed a set of additional signals coresponding to an β-D-glucopyranosyl unit. The linkage position of this glucose was elucidated as follows. Compound **2** showed fragment ions in the negative FAB mass spectrum at m / z 673[M-part'b']$^-$, 153[2,3-dihydroxy benzoyloxy]$^-$ and characteristic 315[153+Glc]$^-$, compared against 511[M-part 'b']$^-$ and 153[2,3-dihydroxy bnezoyloxy]$^-$ for compound **1** (Fig.1). This suggested that additional glucose should be attached to the 2,3-dihydroxy bnezoyl group. Furthermore, the downfield shift effects for the carbons of aromatic ring in **2** [C-2(+1.61 ppm), C-4(+2.75 ppm) and C-6(+2.90 ppm)] when compared against **1** revéaled that the glucose was attached to the C-3 position of the 2,3-dihydroxy benzoyl group. And this was finally confirmed by comparison of the ^1H and ^{13}C NMR signals with those of gelidoside and gentomoside from *Gentiana gelida* which has a 2-hydroxy-3-O-β-D-glucopyranosyl benzoyl unit in the molecule [6] . Therefore, the structure of **2** was determined as shown in Figure 1.

Gentiournoside C(3) exhibited same quasi-molecular ion peak with **2** in the negative FAB mass spectrum. But its ^1H and ^{13}C NMR signals were similar to those of **1** except for a set of signals corresponding to a β-D-glucopyranosyl unit. In the ^{13}C NMR spectrum of **3** due to sugar moiety part, the typical signals at δ80.80, 76.9 and 76.3 were very characteristic of the case of 4-O-glycosylated glucoside [7] , indicating the additional glucose was attached to the hydroxy group of C-4 of the glucose of part 'a' or part 'b'. Since the fragment ions occurred at m / z 673[M-part 'b'] and 153[2,3-dihydroxy benzoyloxy]$^-$ in the negative FAB mass spectrum(Fig. 1), the additional glucose should be attached to the glucosyl group of part 'a'. From the above results, the structure of **3** was established.

Gentiournosides D(4) and E(5) showed quasi-molecular ion peaks at m / z 511[M($C_{23}H_{28}O_{13}$)-H]$^-$ and 673[M($C_{29}H_{38}O_{18}$)-H]$^-$, respectively in the negative FAB mass spectra. On comparison of its ^1H and ^{13}C NMR signals with those of compounds **1**—**3** and 7-O-acetyl loganic acid [8] , **4** was readily assigned as shown in Figure 1, which has a partial structure of **1.** Compared to **4, 5** displayed a set of signals corresponding to an additional esterified β-D-glucopyranosyl unit, for the anomeric carbon signal resonated at realtively upfield δ95.57, while the anomeric proton signal being at relatively downfield δ5.54(d,J = 7.9Hz). Meanwhile, it was also observed that the signal of C-11 was displaced from δ171.40 to δ167.42 when compared against **4.** Thus, the additional glucose should be attached to the carboxyl group of C-11 and the structure of 5 was shown in Figure 1.

It is noted that all the glycosides isolated from *G. urnula* possess a 2,3-dihydroxy benzoyl unit or its derivative in the molecule. This chemical phenomenon may be useful for the chemotaxonomy of this genus.

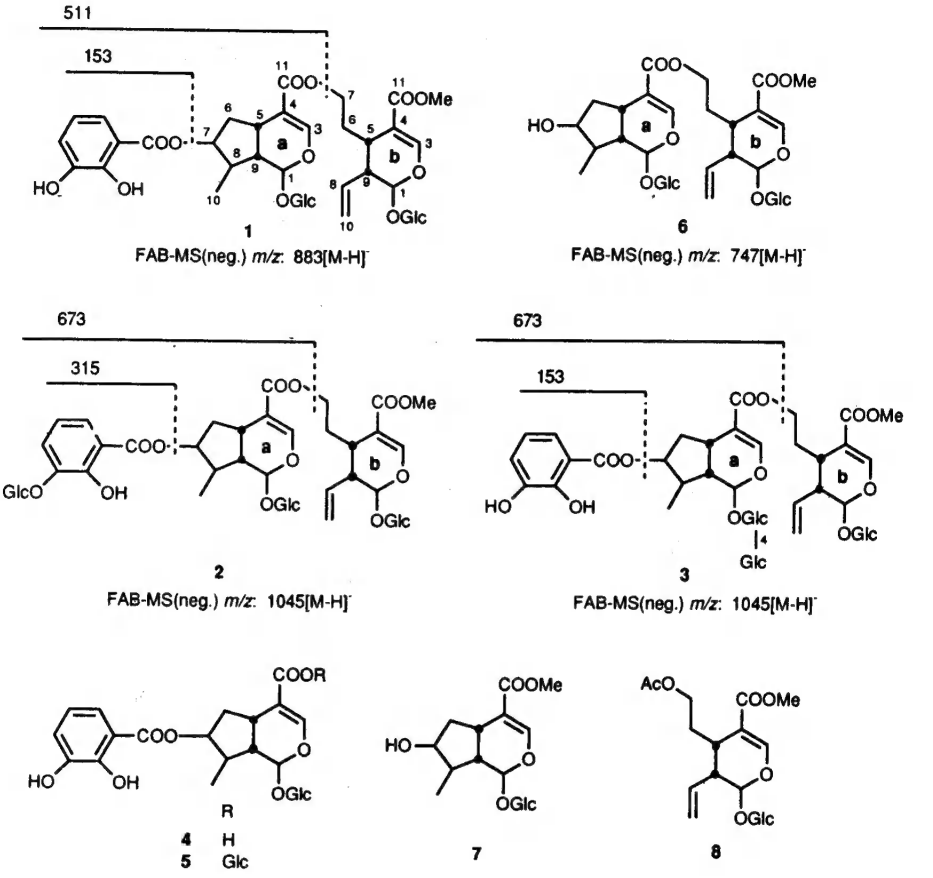

Figure 1. The structures of gentiournosides A-E(**1-5**) and related compouds **6-8**

EXPERIMENTAL

^1H and ^{13}C NMR(DEPT) spectra were recorded in CD_3OD at 400 MHz and 100 MHz, respectively, using TMS as int. standard. Column chromatography was carried out with silica gel (200—300 mesh)and LiChroprep RP-8 (40—63 mesh, Merck). TLC was conducted on precoated Kieselgel 60 F_{254}HPTLC plates (0.2 mm, Merck) and detected by spraying it with 10% H_2SO_4followed by heating.

Plant material. The whole plant of *Gentiana urnula* Smith was collected in Lasa, Tibet at Alt. of 4800 m by Prof. J. S. Yang and identified by Prof. C. Y. Wu. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Table 1 ^{13}C NMR spectral data of compounds 1—8 in CD_3OD(ppm)

	7 [3]	8 [4] *	6	1	2	3	4	5
Part'a'								
C-1	97.81		97.71[a]	97.61[a]	97.71[a]	97.59[a]	97.22	97.62
3	152.22		152.04	152.67	152.73	152.52	149.80	154.16
4	114.06		114.25	113.23	113.23	114.19	116.44	112.62
5	32.24		31.95	32.64	32.72	32.57	33.24	32.49
6	42.79		42.72	40.48	40.55	40.51	40.46	40.20
7	74.77		75.05	80.01	80.34	80.01	80.22	80.02
8	42.24		42.07	41.15	41.19	41.16	41.13	41.12
9	46.55		46.58	47.21	47.20	47.29	47.56	47.31
10	13.61		13.30	13.73	13.64	13.77	13.82	13.68
11	169.60		169.01[b]	168.83[b]	168.84[b]	168.80[b]	171.40	167.42
OMe	51.85							
1-O-Glc-1	100.11		100.14	100.18[c]	100.24[c]	100.22	100.25	100.37
2	75.12		74.76[c]	74.64[d]	74.87[d]	74.51[c]	74.86	74.78
3	78.04		78.02[d]	78.01[e]	77.99[e]	76.85[d]	78.04	78.05[a]
4	71.62		71.59	71.62	71.63[f]	80.80	71.71	71.65[b]
5	78.36		78.35[d]	78.30[e]	78.29[e]	76.32[d]	78.32	78.37[a]
6	62.48		62.78	62.62	62.86[g]	62.05	62.88	62.84[c]
Glc-1						104.39		95.57
2						74.65[c]		74.07
3						78.04[e]		78.11[a]
4						71.48[f]		71.16[b]
5						78.32[e]		78.73[a]
6						62.55		62.46[c]
Patr'b'								
1		97.8	97.71[a]	97.75[a]	97.79[a]	97.80[a]		
3		153.6	153.59	153.64	153.70	153.65		
4		111.5	111.46	111.48	111.50	111.51		
5		31.4	30.82	31.26	31.32	31.34		
6		30.0	29.85	30.06	30.14	30.14		
7		64.2	63.39	63.59	63.64	63.66		
8		135.6	135.54	135.56	135.61	135.59		
9		45.4	45.18	45.25	45.24	45.28		
10		119.5	119.70	119.56	119.63	119.62		
11		169.2	169.21[b]	169.19[b]	169.19[b]	169.19[b]		
OMe		51.7	51.81	51.82	51.95	51.87		
Glc-1		100.2	100.14	100.30[c]	100.34[c]	100.22		
2		74.7	74.62[c]	74.77[d]	74.79[d]	74.95[c]		
3		78.1	77.91[d]	77.91[e]	77.68[e]	78.04[e]		
4		71.6	71.59	71.62	71.63	71.66[f]		
5		78.4	78.25[d]	78.30[e]	78.29[e]	78.32[e]		
6		62.8	62.78	62.62	62.86	62.55		
OAc		20.8						
		172.9						
Ar-1				114.15	115.10	114.10	114.28	114.17
2				151.40	153.01	151.42	151.60	151.40
3				147.11	147.28	147.16	147.13	147.14
4				121.85	124.60	121.68	121.74	121.69
5				120.12	120.22	120.14	120.04	120.16
6				121.15	124.05	121.20	121.28	121.20
Ar-C=O				171.27	170.81	171.29	171.40	171.31
Glc-1					103.26			
2					74.66[d]			
3					77.99[e]			
4					71.36[f]			
5					78.29[e]			
6					62.58[g]			

* In this reference, the signals of C-5 and C-6 of aglycone moiety were reversed. According to our DEPT ^{13}C NMR, they should be revised as shown here. a-g: Signals in each vertical column can be interchangeable.

Table 2 ^{1}H NMR spectral data of compounds 1—8 in CD_3OD(ppm)*

	7 [3]	8 [4]	6	1	2	3	4	5
Part'a'								
H-1	5.26 d (4.5)		5.29 d (3.8)	5.34 d (4.4)	5.39 d (4.4)	5.30 d (4.2)	5.28 d (4.6)	5.38 d (3.4)
3	7.38 d (1.2)		7.38 s[a]	7.43 s[a]	7.45 s[a]	7.43 s[a]	7.29 s	7.62 s
5	3.12 m		3.10 m	3.25 m	3.25 m	3.25 m	3.32 m	3.24 m
6a	1.62 m		1.65 m	1.90 m	1.96 m	1.90 m	1.93 m	1.96 m
6b	2.03 m		2.05 m[b]	2.50 m	2.50 m	2.45 m	2.44 m	2.47 m
7	4.04 m		* *	5.50 m	5.46 m	5.46 m	5.45 m	5.46 m
8	1.87 m		1.84 m[c]	2.23 m	2.23 m	2.21 m	2.16 m[a]	2.23 m
9	2.23 m		2.22 m	2.23 m	2.23 m	2.21 m	2.24m[a]	2.23 m
10	1.09 d (6.9)		1.09 d (6.8)	1.15 d (6.7)	1.15 d (6.8)	1.15 d (6.8)	1.13 d (6.8)	1.16 d (6.7)
OMe	3.69 s							
1-O-Glc-1	4.64 d (7.8)		4.66 d[d] (7.9)	4.70 d[b] (8.0)	4.69 d[b] (8.2)	* *	4.69 d (7.2)	4.71 d (7.6)
Glc-1						* *		5.54 d (7.9)
Patr'b'								
1		5.52 d (6.5)	5.54 d (5.9)	5.53 d (6.3)	5.53 d (6.3)	5.53 d (6.3)		
3		7.44 s	7.46 s[a]	7.46 s[a]	7.47 s[a]	7.45 s[a]		
5		2.85 dd (12.5,6)	2.92 m	2.92 m	2.92 m	2.90 m		
6a		1.93 dt (16,6)	2.03 m[b]	2.03 m	2.01 m	1.96 m		
6b		1.81 ddd (16,12.5,6)	1.77 m[c]	1.80 m	1.80 m	1.82 m		
7		4.08 m	4.20 m	4.19 m	4.18 m	4.16 m		
8		5.77 ddd (17,10,8.5)	5.74 ddd (17.8,11,8)	5.77 ddd (17.2,10.6,8)	5.75 ddd (17.3,11,8)	5.77 m		
9		2.66 ddd (8.5,6.5,6)	2.68 m	2.65 m	2.63 m	2.64 m		
10a		5.30 dd (17,1.5)	5.31 d (17.8)	5.28 d (17.2)	5.27 d (17.3)	5.28 d (18.0)		
10b		5.25 dd (10,1.5)	5.25 d (11.1)	5.22 d (10.6)	5.20 d (10.7)	5.22 d (10.4)		
OMe		3.68 s	3.67 m	3.68 s	3.71 s	3.68 s		
Glc-1		4.69 d (8)	4.69 d[d] (7.9)	4.72 d[b] (8.0)	4.72 d[b] (9.0)	* *		
OAc		2.01 s						
Ar-4				7.04 dd (7.8,1.3)	7.54 d (7.9)	7.05 d (8.0)	7.02 d (7.5)	7.04 d (7.8)
5				6.78 t (7.9)	6.90 t (7.9)	6.78 t (8.0)	6.75 t (7.4)	6.77 t (7.8)
6				7.34 dd (7.9,1.4)	7.43 d (7.9)	7.38 d (8.0)	7.35 d (7.4)	7.34 d (7.8)
Glc-1					* *			

* The coupling constants are expressed in Hz in paratheses.

* * The coupling patterns were overlapped, a—d Signals in each vertical column can be interchangeable.

Extraction and isolation. Dried whole herb (1.0 kg) was extracted with MeOH under reflux. After re-

moval of solvent by evapn, the combined extracts (213 g) was suspended in H_2O and extracted successively with Et_2O, $CHCl_3$ and n-BuOH. The n-BuOH layer was evapd under *in vacuo* to give a residue (44 g), which was subjected to silica gel eluting with $CHCl_3$-MeOH(9 : 1 to 2.8 : 1) to give frs. 1—3. Fr. 1 was repeatedly chromatographed on silica gel with $CHCl_3$-MeOH-H_2O to yield **1**(350 mg). Fr. 2 was repeatedly chromatographed on reversed phase silica gel with 54% MeOH to give **3**(30 mg) and **4**(15 mg). Fr.3 was separated by repeated column chromatography on reversed phase silica gel 52% MeOH and silica gel with $CHCl_3$-MeOH-H_2O(55 : 10 : 1) to yield **2**(150 mg) and **5**(200 mg).

Gentiournoside A(1) Powder, $[\alpha]_D^{23}$-62° (MeOH; c 0.216); UVλ_{max}^{EtOH}nm(lgε): 222(3.47), 238(3.35), 322(2.40); IRν_{max}^{KBr}cm^{-1}: 3400(br), 1700, 1670, 1625, 1580, 1460, 1300, 750; FAB-MS(neg.) m/z: 883[M-H]$^-$, 721[M-Glc-H]$^-$, 511[M-part 'b']$^-$, 153[2,3-dihydroxy benzoyloxy]$^-$, 137[153-O]$^-$.

Gentiournosides B(2) Power, $[\alpha]_D^{26}$-81° (MeOH; c 0.268); UVλ_{max}^{EtOH}nm (lgε):213(4.49), 239(4.41), 314(3.61); IRν_{max}^{KBr}cm^{-1}: 3400(br), 1700, 1670, 1625, 1585, 1460, 1300, 750; FAB-MS(neg.) m/z: 1045[M-H]$^-$, 883[M-Glc-H]$^-$, 673[M-part 'b']$^-$, 315[2-hydroxy 3-O-glucosyl benzoyloxy]$^-$, 153, 137.

Gentiournosides C(3) Power, $[\alpha]_D^{26}$-110° (pyridine; c 0.230); UVλ_{max}^{EtOH}nm (lgε): 221(4.45), 235(4.34),325(3.65); IRν_{max}^{KBr}cm^{-1}: 3400(br), 1695, 1665, 1625, 1585, 1460, 1300, 753; FAB-MS(neg.)m/z: 1045[M-H]$^-$, 883[M-Glc-H]$^-$, 721[M-Glc×2-H]$^-$, 673[M-part 'b']$^-$, 537[M-part 'b'-2,3-dihydroxy benzoyl]$^-$, 511[M-part 'b'-Glc]$^-$, 153, 137.

Gentiournosides D(4) Power, $[\alpha]_D^{26}$-55° (MeOH; c 0.211); UVλ_{max}^{EtOH}nm (lgε): 217, 247, 327; IRν_{max}^{KBr}cm^{-1}: 3400(br), 1662(br), 1613, 1585, 1460, 1300, 1070, 750; FAB-MS(neg.) m/z: 511[M-H]$^-$, 349[M-Glc-H]$^-$, 153, 137.

Gentiournosides E(5) Power, $[\alpha]_D^{26}$-21° (MeOH; c 0.189); UVλ_{max}^{EtOH}nm (lgε): 220(4.37), 244(4.20), 323(3.48); IRν_{max}^{KBr}cm^{-1}: 3400(br), 1705, 1624, 1626, 1585, 1462, 1300, 751; FAB-MS(neg.)m/z: 673[M-H]$^-$, 511[M-Glc-H]$^-$, 153, 137.

Alkaline hydrolysis of 1 A soln of **1** (113 mg) in 0.5% Et_2NH-MeOH(2 mL) was kept at 50° for 40 h. The reaction mixt. was neutralized with Amberlite MB-3 and concd to dryness to give a residue, which was chromatographed on silica gel with $CHCl_3$-MeOH-H_2O(7 : 3 : 1; lower layer) to yield compound **6**(27 mg) with a recovery of **1**(55 mg). **6**: Powder, $[\alpha]_D^{16}$-88° (MeOH; c 0.573); FAB-MS(neg.)m/z: 747[M-H]$^-$, 585[M-Glc-H]$^-$.

Acknowledgements The authors are grateful to Dr. Royji Kasai (Hiroshima University School of Medicine, Japan) for performing negative FAB mass spectra, Prof. J. S. Yang for collecting plant materials and the staff of the Instrument Group of our Lab for UV, IR and NMR spectral measurements.

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